APPENDIX A

Pending claims after entry preliminary amendment

Re: USSN 09/375,609
For: METHODS AND KITS FOR OBTAINING AND ANALYZING SKIN SAMPLES FOR THE DETECTION OF BIOLOGICAL FACTORS (amended)
Applicant: Rheins and Morhenn
Filed: Aug. 17, 1999
F&R Ref: 09373-002001

ELECTED RESTRICTION GROUP I:

I. Claims 1-10 [new claims 64 to 102], drawn to a method of obtaining a biological factor from cells below the stratum corneum of the skin of a subject.

[canceled claims 1 to 10: upon cancellation of claims 1 to 10, Group I will include new claims 64 to 102 as set forth below):

11. (as filed) A method of distinguishing an irritant contact dermatitis (ICD) from an allergic contact dermatitis (ACD) in a subject, comprising, quantifying a polynucleotide level encoding a cytokine, wherein the polynucleotide level determines whether the dermatitis is ICD or ACD.

12. (as filed) The method of claim 11, wherein the polynucleotide is RNA or DNA.

13. (as filed) The method of claim 12, wherein the RNA is mRNA.

14. (as filed) The method of claim 11, wherein the subject is a human.

15. (as filed) The method of claim 11, wherein the polynucleotide is from the cells below the stratum corneum of the skin, the method further comprising:

(a) removing the stratum corneum; and

(b) collecting polynucleotide from the surface exposed after removal of the stratum corneum.

16. (as filed) The method of claim 15, wherein removal of the stratum corneum uses procedures selected from the group consisting of:

(a) abrading the stratum corneum; and

(b) contacting the stratum corneum with an adhesive surface.

17. (as filed) The method of claim 15, wherein the polynucleotide is collected from the surface exposed after removal of the stratum corneum using a procedure selected from the group consisting of:

(a) scraping the surface exposed with a rigid surface; and

(b) contacting the surface exposed with an adhesive surface.

18. (as filed) The method of claim 17, wherein the adhesive surface comprises adhesive tape.

19. (as filed) The method of claim 13, wherein the mRNA is specific for a cytokine.

20. (as filed) The method of claim 19, wherein the cytokine is IL-4 and IL-8.

21. (as filed) The method of claim 20, wherein the absence of IL-4 in the presence of a reaction is characteristic of ICD.

22. (as filed) The method of claim 20, wherein the level of increase in IL-8 is indicative of the severity of ICD.

23. (as filed) The method of claim 19, wherein the cytokine is IL-4.

24. (as filed) The method of claim 23, wherein an increase in IL-4 is characteristic of ACD.

25. (as filed) The method of claim 24, wherein the level of increase in IL-4 is indicative of the severity of ACD.

26. (as filed) The method of claim 11, further comprising exposing the skin to a factor prior to isolating the polynucleotide.

27. (as filed) The method of claim 26, wherein the factor is an irritant, antigen or allergen.

28. (Amended) A method of diagnosing irritant contact dermatitis (ICD) in a subject, comprising quantifying a polynucleotide encoding a cytokine selected from the group consisting of IL-4 and IL-8 in cells isolated from the subject, wherein the amount of IL-4 or IL-8 is indicative of ICD.

29. (as filed) The method of claim 28, wherein the polynucleotide is detected by PCR.

30. (as filed) The method of claim 28, wherein the polynucleotide is detected by hybridization with a polynucleotide probe.

31. (as filed) The method of claim 28, wherein the polynucleotide is detected by RNase protection assay.

32. (as filed) The method of claim 28, wherein the cells are skin cells.

33. (as filed) The method of claim 28, wherein the subject is a mammal.

34. (as filed) The method of claim 33, wherein the mammal is a human.

35. (Amended) A method of diagnosing allergic contact dermatitis (ACD) in a subject, comprising quantifying a polynucleotide encoding IL-4 in cells of the subject, wherein an elevated amount of IL-4 is indicative of ACD.

36. (as filed) The method of claim 35, wherein the IL-4 is detected by PCR.

37. (as filed) The method of claim 35, wherein the IL-4 is detected by hybridization with a polynucleotide probe.

38. (as filed) The method of claim 35, wherein the IL-4 is detected by RNase protection assay.

39. (as filed) The method of claim 35, wherein the cells are skin cells.

40. (as filed) The method of claim 35, wherein the subject is a mammal.

41. (as filed) The method of claim 40, wherein the mammal is a human.

42. (as filed) A method of identifying a compound which causes a dermatitis, comprising contacting a section of skin with the compound under conditions which would induce a dermatitis and detecting a polynucleotide encoding a cytokine wherein the presence of the polynucleotide is indicative of a dermatitis.

43. (as filed) The method of claim 42, wherein the compound is an allergen.

44. (as filed) The method of claim 42, wherein the compound is an irritant.

45. (as filed) The method of claim 42, wherein the dermatitis is allergic contact dermatitis (ACD).

46. (as filed) The method of claim 42, wherein the dermatitis is irritant contact dermatitis (ICD).

47. (as filed) The method of claim 42, wherein the skin is contacted in vivo.

48. (as filed) The method of claim 42, wherein the skin is contacted in vitro.

49. (as filed) The method of claim 42, further comprising isolating polynucleotides from the skin.

50. (as filed) The method of claim 49, wherein the polynucleotides are DNA or RNA.

51. (as filed) The method of claim 50, further comprising quantifying a polynucleotide encoding IL-4, wherein an elevated amount of IL-4 is indicative of ACD.

52. (as filed) The method of claim 50, further comprising quantifying a polynucleotide encoding a cytokine selected from the group consisting of IL-4 and IL-8 in cells isolated from the subject, wherein the amount of IL-4 or IL-8 is indicative of ICD.

53. (as filed) The method of claim 52, wherein an increase in IL-8 in the absence of IL-4 is indicative of ICD.

54. (Amended) A method of diagnosing allergic contact dermatitis (ACD) in a subject, comprising quantifying a polynucleotide encoding IL-13 in cells of the subject, wherein an elevated amount of IL-13 is indicative of ACD.

55. (as filed) The method of claim 54, wherein the IL-13 is detected by PCR.

56. (as filed) The method of claim 54, wherein the IL-13 is detected by hybridization with a polynucleotide probe.

57. (as filed) The method of claim 54, wherein the IL-13 is detected by RNase protection assay.

58. (as filed) The method of claim 54, wherein the cells are skin cells.

59. (as filed) The method of claim 54, wherein the subject is a mammal.

60. (as filed) The method of claim 59, wherein the mammal is a human.

61. (as filed) A kit for obtaining polynucleotides from the skin, the kit comprising:

a cell collection device selected from the group consisting of a rigid surface and an adhesive tape; and

a cell lysis buffer suitable of preserving polynucleotides or a computer chip suitable for preserving polynucleotides.

62. (as filed) The kit of claim 61, which further comprises an mRNA detection reagent.

63. (as filed) A kit for distinguishing an irritant reaction from an allergic reaction, the kit comprising a cell collection device, a cell lysis buffer, an mRNA detection reagent.

New claims added in preliminary amendment:

64. A non-invasive method for obtaining a skin sample for use in isolating or detecting a nucleic acid in a skin sample, the method comprising:

(a) applying at least one application of an adhesive to the skin and removing the adhesive from the skin such that a sample comprising a nucleic acid adheres to the adhesive after its removal, or, scraping the skin with an instrument to remove a sample comprising a nucleic acid from the skin, thereby obtaining a skin sample comprising a nucleic acid;

(b) isolating or detecting the nucleic acid from the skin sample of step (a).

65. The method of claim 64, wherein the skin sample consists essentially of stratum corneum.

66. The method of claim 64, wherein the skin sample consists essentially of stratum lucidum cells.

67. The method of claim 64, wherein the skin sample consists essentially of stratum granulosum cells.

68. The method of claim 64, wherein the skin sample consists essentially of stratum spinosum cells.

69. The method of claim 64, wherein the skin sample consists essentially of stratum basilis cells.

70. The method of claim 64, wherein an adhesive surface is applied one time to the skin.

71. The method of claim 70, wherein an adhesive surface is applied two or more times to the skin.

72. The method of claim 65, wherein the stratum corneum skin sample is isolated by one application of an adhesive surface to an outer layer of the skin.

73. The method of claim 64, wherein the adhesive surface comprises an adhesive tape.

74. The method of claim 73, wherein the adhesive tape comprises a duct tape, a Scotch™ tape or a D-SQUAME™ tape.

75. The method of claim 64, wherein a skin sample is isolated by scraping an outer layer of skin with a rigid instrument.

76. The method of claim 64, wherein the nucleic acid comprises a DNA.

77. The method of claim 64, wherein the nucleic acid comprises an RNA.

78. The method of claim 77, wherein the RNA comprises an mRNA.

79. The method of claim 78, wherein the nucleic acid encodes a polypeptide.

80. The method of claim 79, wherein the polypeptide comprises a cytokine.

81. The method of claim 79, wherein the polypeptide comprises an interleukin.

82. The method of claim 79, wherein the cytokine comprises an IL-1, an IL-2, an IL-3, an IL-4, an IL-5, an IL-6, an IL-7, an IL-8, an IL-9, an IL-10, an IL-12, an IL-13, an IL-14, a granulocyte macrophage colony stimulating factor (GM-CSF), or an interferon.

83. The method of claim 78, wherein the polypeptide comprises an inflammatory mediator.

84. The method of claim 83, wherein the inflammatory mediator comprises a leukotriene or a prostaglandin.

85. The method of claim 64, further comprising identifying or quantifying the nucleic acid.

86. The method of claim 85, wherein identifying or quantifying the nucleic acid is by a polymerase chain reaction (PCR).

87. The method of claim 85, wherein identifying or quantifying the nucleic acid is by hybridization with a polynucleotide probe.

88. The method of claim 85, wherein identifying or quantifying the nucleic acid is by RNase protection assay.

89. The method of claim 85, wherein by identifying or quantifying a nucleic acid in a recovered sample the presence of a local or systemic disease, a disorder, a genetic disease, or an inflammatory reaction is identified, distinguished, or diagnosed.

90. The method of claim 64, wherein the nucleic acid is associated with a local biological reaction.

91. The method of claim 64, wherein the nucleic acid is associated with a systemic biological reaction.

92. The method of claim 64, further comprising applying the sample to a chip.

93. The method of claim 64, wherein the skin sample is a human skin sample.

94. The method of claim 64, further comprising applying the cellular material sample to a chip.

95. A non-invasive method for isolating a nucleic acid in a skin cell of a subject comprising:

a) removing an outer skin layer to expose an inner skin layer by scraping or stripping by use of an adhesive;

(b) removing an inner skin sample from the exposed skin by scraping or stripping by use of an adhesive; and,

(c) isolating or detecting a nucleic acid sample from the inner skin sample.

96. The method of claim 95, wherein the outer skin layer comprises a stratum corneum.

97. The method of claim 95, wherein the adhesive comprises an adhesive tape.

98. The method of claim 95, wherein the nucleic acid comprises a DNA.

99. The method of claim 95, wherein the nucleic acid comprises an RNA.

100. The method of claim 99, wherein the nucleic acid encodes a polypeptide.

101. The method of claim 95, further comprising identifying or quantifying the nucleic acid.

102. The method of claim 95, further comprising applying the nucleic acid, or complementary equivalent, to a chip.

7 103 ~~102~~. The method of claim 95, wherein the skin sample is a human skin sample.